Serologic Diagnosis of Syphilis

Use of the *Treponema pallidum* Immobilization (TPI) Test and the Fluorescent Treponemal Antibody (FTA) Test

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■ *Comparative data from four different laboratories on the TPI and FTA tests on VDRL-reactive sera from patients with no symptoms of syphilis showed agreement of results of the FTA test with those obtained with the TPI test (the reference test) varying from 71 per cent to 112 per cent.*

The FTA test is complex and requires additional research before its use as a routine public health laboratory procedure can be recommended. The TPI test appears to be unequivocally the test of choice for the diagnosis of late and latent syphilis.

FIFTY YEARS AGO Wassermann described a complement-fixation test for syphilis in which tissue extracts of human fetal syphilitic livers containing *Treponema pallidum* were employed as the antigen. In this last half century thousands of patients have been treated for syphilis who did not have the disease and, on the other hand, a lesser number with the disease have remained untreated due to the great confusion in its serologic diagnosis. Yet, today we are still searching for a simple dependable test for the laboratory diagnosis of syphilis. Significant progress has been made, however. With the development of the *Treponema pallidum* immobilization (TPI) test in 1949 by Nelson and Mayer,[10] the shortcomings of the non-treponemal tests became evident, and it is now known that they serve only as screening tests. During the last decade in our laboratory the percentage of biologic false positive (BFP) reactors among a total of 32,000 TPI tests has increased from 54 per cent to 71 per cent,[4] which is indicative of the magnitude of the problem.

Since the TPI test was described, 22 other treponemal tests have been developed. In general, each has been reported as a simple and inexpensive procedure with sensitivity and specificity equivalent to that of the TPI test. The majority of such tests have been short lived, however, when subjected to the rigor of routine use in the public health laboratory.

The latest developments in the treponemal tests are a series of fluorescent treponemal antibody (FTA) tests. They are modifications of the original FTA test described by Deacon, Falcone and Harris,[5] in which non-living *T. pallidum* is mixed with the patient's serum on a slide, following which an anti-human globulin labeled with fluorescein isothiocyanate is applied. In the presence of antibody, the spirochetes exhibit a fluorescence when observed with a fluorescent microscope and a special fluorescent lamp.

The present report includes preliminary studies with the FTA in our laboratory and a review of comparative recent studies of the TPI and FTA tests by three other investigators.[3,7,9,12] employing

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VDRL* reactive serum from patients with no clinical manifestations of syphilis.

Outline of TPI and FTA Tests

To date no simple treponemal test has been developed. The TPI test requires maintenance of a virulent *T. pallidum* infection in VDRL negative rabbits, harvesting of the spirochetes and subsequent suspension in a special medium. The spirochetes must be maintained free from contamination and under essentially anaerobic conditions at all times. Positive reaction is determined by immobilization of *T. pallidum*, as observed under the darkfield microscope 18 hours after incubation of the patient's serum with the spirochetes *in vitro*, in the presence of complement. With proper controls, of 25 spirochetes counted with a darkfield microscope, the numbers of motile and non-motile organisms are determined. The ratio of motile to non-motile organisms is the basis for the interpretation of the test.

The FTA test requires a suspension of killed virulent *T. pallidum* which is mixed on a slide with the patient's serum and subsequently an anti-human globulin that is labeled with fluorescein isothyiocyanate is added. After special fixation and fluorescent staining, the slide is examined for spirochetes coated with fluorescent antibody. A count is made of the spirochetes. Those that show no fluorescence and 1+ fluorescence are considered non-reactive, while those that are fluorescent to a degree designated as 2+, 3+ and 4+ are considered reactive. In the FTA-200 test the serum is diluted 1:200 and in the FTA-ABS (absorbed) the serum is diluted 1:5 with a heavy suspension of sonically disrupted *T. pallidum*, Reiter strain.

From these brief descriptions, one can readily appreciate that the TPI and FTA tests are for well-trained personnel only.

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Present Study

The TPI test described by Nelson and Mayer,[10] with the modifications of Magnuson and Thompson,[8] Thompson and Magnuson[11] and by Boak and Miller[1] now in use in our laboratory for ten years, was employed. The FTA test was carried out in four different laboratories according to the method recommended in *Laboratory Procedures for Modern Syphilis Serology*, 1962.[6] Fluorescent spirochetes (as microscopically observed) varying from 2+ to 4+ intensities were reactive and those ranging from no fluorescence to a 1+ fluorescence were considered non-reactive.

One hundred four specimens of serum from patients reactive to VDRL test and considered to be "diagnostic problem sera" were subjected to the TPI and FTA tests. The sera were received from the Los Angeles County Health Department and from physicians throughout the State of California.

Results

Thirty-five (33.6 per cent) of the 104 sera were reactive to the TPI test and 69 (66.4 per cent) were non-reactive. Twenty-nine (27.9 per cent) were reactive with the FTA test and 75 (72.1 per cent) were non-reactive. Thus, only 29 (82.9 per cent) of the 35 TPI-reactive sera and 65 (94.2 per cent) of the 69 TPI non-reactive sera were respectively reactive and non-reactive by the FTA test.

The comparative data on the TPI and FTA tests by the four laboratories are representative of recent studies on sera from patients with reactive non-treponemal tests submitted for the TPI test. The results of the reactive TPI tests range from 33.6 per cent by Laboratory II to 57 per cent by Laboratory III. The percentages in the FTA tests range from 28.4 per cent by Laboratory I to 56 per cent by Laboratory IV employing the FTA-ABS test (Table 1). In the case of the two laboratories, III

TABLE 1.—*Comparative Data from Four Laboratories on TPI and FTA Tests on STS-Reactive Specimens*

Year	Investigator	Laboratory	Number of Specimens		TPI Number	TPI Per Cent	FTA Number	FTA Per Cent	Per Cent Agreement of FTA with TPI
1961	Wilkinson[12]	I	144	R	58	40.2	41	28.4	71
				NR	86	59.8	103	71.6	
1963	Miller, Whang, Carpenter[9]	II	104	R	35	33.6	29	27.9	83
				NR	69	66.4	75	72.1	
1963	Leibovitz, Oberhofer, Meachan, and Diestelhorst[7]	III	705	R	433	57	367†	52	95
				NR	332	43	338†	48	
1964	Bradford, Bodily, Puffer, Ketterer[3]	IV	200	R	99*	50	77†	39	77
				NR	92	46	123	61	
			200	R	99*	50	111‡	56	112
				NR	92	46	89	44	

*9 sera inconclusive. †FTA-200 test. ‡FTA-ABS test.
R=Reactive. NR=Non-reactive.

and IV, that used the FTA-200, the percentage of reactors was 52 per cent for the former and 39 per cent for the latter. Agreement of the FTA-200 and FTA-ABS with the TPI test in the four different laboratories was respectively 71 per cent, 83 per cent, 77 per cent (FTA-200), 95 per cent (FTA-200), 112 per cent (FTA-ABS). The specificity of the tests was more uniform.

Discussion

Deacon, Falcone and Harris[5] described the FTA test for syphilis in 1957, and several modifications of the test have been recommended during the last four years. The FTA-ABS modification of the test appears to have some promise, but further research and experience are essential before it can be accepted as an approved procedure for the diagnosis of syphilis. The TPI test, on the other hand, has been in use for ten years and a background of knowledge and its dependability has been established. In our laboratory the correlation of the results of the TPI test with the clinical status of the patient has been a most gratifying experience. This is especially borne out by the fact that among over 1,000 untreated BFP pregnant women with reactive VDRL sera, none delivered a syphilitic infant. No doubt errors in testing 32,000 sera have occurred but none has come to our attention and hundreds of these cases have been discussed with the patients' physicians.

A comparison of the results of TPI tests carried out in other laboratories has been difficult because of the use of different media for suspending and maintaining the spirochetes, the use of either fresh or lyophilized complement and finally because of counting the spirochetes and interpretation of the test. No standardized technique has been established. Likewise, the FTA tests are not uniformly carried out, especially as they are in a developmental stage.

With few exceptions, reports of the FTA tests have been made on sera from so-called normal persons and on sera from patients with early and late syphilis. Here again it is difficult to evaluate the data because of differences in the selection of cases, and lack of standardized technique. Such tests are necessary but in this report we are concerned with the routine comparative use of the test on so-called problem sera submitted for the differentiation of BFP reactors for syphilis from those due to specific infection with *T. pallidum*. Early syphilis in most instances can be recognized and treated effectively. The main problem is to lift the stigma of syphilis from persons with reactive serologic tests for syphilis who do not have the disease.

In most reports the FTA is designated as a simple procedure. In our limited experience the test has not proven so. The major problems with the FTA test include adherence of sufficient number of *T. pallidum* to the slide, non-specific fluorescence, unavailability of satisfactory anti-human globulin and the necessity of its rigid laboratory control over each lot. Fluorescent microscopy likewise requires experience, expensive equipment and special training. The interpretation of tests is blurred by subjectivity of the microscopist. Another inherent difficulty is the differentiation of *T. Pallidum* from detached fluorescent coils in the tails of spermatozoa. Leibovitz, Oberhofer, Meachan and Diestelhorst[7] recommended performing the FTA test on paired specimens, which requires duplication of effort.

In our opinion the test of choice remains the TPI test even though it may be slightly more expensive. Its record for sensitivity and reproducibility of results over the past decade make it the dependable reference test for syphilis.

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REFERENCES

1. Boak, R. A., and Miller, J. N.: A simple medium for maintaining the viability of *Treponema pallidum* in the *Treponema pallidum* immobilization test, Am. J. Syph., 38:429-433, 1954.

2. Boak, R. A., Carpenter, C. M., Miller, J. N., Drusch, H. E., Chapman, J. M., and Heidbreder, G. A.: Biologic false-positive reactions for syphilis in pregnancy as determined by *Treponema pallidum* immobilization test., Surg. Gyn., Obs., 101:751-752, 1955.

3. Bradford, L. L., Bodily, H. J., Puffer, J., and Ketterer, W. Evaluation of fluorescent treponemal antibody (FTA-200 and FTA-absorption) and *Treponema pallidum* immobilization (TPI) tests in the serodiagnosis of syphilis. Presented before the 14th Annual Symposium on Recent Advances in the Study of Venereal Diseases, Houston, Texas, January 24, 1964.

4. Carpenter, C. M., Boak, R. A., Le Clair, R. A., and Miller, J. N.: The increasing incidence of BFP reactors for syphilis among 30,000 sera subjected to the TPI test during the last decade. (To be published.)

5. Deacon, W. E., Falcone, V. H., and Harris, A.: A fluorescent test for treponemal antibodies, Proc. Soc. Exp. Biol. and Med., 96:477-480, 1957.

6. Laboratory Procedures for Modern Syphilis Serology, 1962 Manual, Public Health Service Publication No. 988, Washington, D. C., U. S. Government Printing Office.

7. Leibovitz, A., Oberhofer, T. R., Meachan, J. T., and Diestelhorst, T. N.: Enhancement of specificity of the fluorescent treponemal antibody test as compared with the TPI test, Am. J. Clin. Path., 40:480-486, 1963.

8. Magnuson, H. F., and Thompson, F. A.: Treponemal immobilization test of normal and syphilitic serums, J. Ven. Dis. Inform., 30:309-320, 1949.

9. Miller, J. N., Whang, S. J., Boak, R. A., and Carpenter, C. M.: Complexities of the fluorescent treponemal antibody (FTA) test and its preliminary evaluation in the serologic diagnosis of syphilis, World Health Organization, WHO/VDT/43, Oct. 9, 1963.

10. Nelson, R. A., Jr., and Mayer, M. M.: Immobilization of *Treponema pallidum in vitro* by antibody produced in syphilitic infection, J. Exp. Med., 89:369-393, 1949.

11. Thompson, F. A., and Magnuson, H. F.: Studies on increasing sensitivity of treponemal immobilization test for syphilis, Amer J. Syph., 35:23-34, 1951.

12. Wilkinson, A. E.: Fluorescent treponemal antibody test. A preliminary report, Brit. J. Ven. Dis., 37:59-63, 1961.